

immune checkpoint blockade, costimulatory molecule...) to preferentially concentrate the cytokine to the targeted antigen-expressing cells. However, the affinity of IL-7 cytokine for its CD127/CD132 receptor (nanomolar to picomolar range) may be higher than the affinity of the antibody for its target. Hence, the cytokine will drive the pharmacokinetics of the product leading to a fast depletion of the available drug *in vivo* due to the target-mediated drug disposition (TMDD) mechanism. This rapid elimination has been described for immunocytokine like IL-2 or IL-15 showing that pharmacokinetic properties of the fusion protein may directly impact on drug performance (List et Neri Clin Pharmacol. 2013; 5(Suppl 1): 29–45). Then, it remains therefore a significant need in the art for new and improved IL-7 variant that allows to improve the distribution and reduce elimination of IL-7 products, particularly of bifunctional molecule comprising IL-7. The inventors have made a significant step forward with the invention disclosed herein.

SUMMARY

The inventors provide IL-7 mutations and optimized Fc backbones in order to improve the distribution and elimination of a bifunctional molecule for an enhanced biological effect *in vivo*. The inventors observed that IL-7 mutations; particularly in combination with the IgG isotype and linker length, allows a better distribution of the bifunctional molecule and a longer half-life *in vivo*.

The bifunctional molecules provided herein particularly demonstrates a good pharmacokinetics and pharmacodynamics *in vivo*, particularly in comparison with bifunctional molecule comprising an IL-7 wild type. In addition, advantageous and unexpected properties have been associated to these new molecules as detailed in the introduction of the detailed description and in the examples.

In a first example, the disclosure relates to a bifunctional molecule comprising an interleukin 7 (IL-7) variant conjugated to a binding moiety, wherein:

- the binding moiety binds to a target specifically expressed on immune cells surface,
- the IL-7 variant presents at least 75% identity with a wild type human IL-7 (wth-IL-7) comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1, wherein the variant comprises at least one amino acid mutation which i) reduces affinity of the IL-7 variant for IL-7 receptor (IL-7R) in comparison to the affinity of wth-IL-7 for IL-7R, and ii) improves pharmacokinetics of the bifunctional molecule comprising the IL-7 variant in comparison with a bifunctional molecule comprising wth-IL-7.

In particular, the at least one mutation is an amino acid substitution or a group of amino acid substitutions selected from the group consisting of (i) C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, or C47S-C92S and C34S-C129S, (ii) W142H, W142F or W142Y, (iii) D74E, D74Q or D74N, iv) Q11E, Y12F, M17L, Q22E and/or K81R; or any combination thereof (i.e., the amino acid numbering being as shown in SEQ ID NO: 1).

In particular, in one aspect, the disclosure concerns bifunctional molecule comprising an interleukin 7 (IL-7) variant conjugated to a binding moiety, wherein:

- the binding moiety binds to a target specifically expressed on immune cells surface,

- the IL-7 variant presents at least 75% identity with a wild type human IL-7 (wth-IL-7) comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1, wherein the variant comprises at least one amino acid mutation selected from the group consisting of (i) W142H, W142F or W142Y, (ii) C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, or C47S-C92S and C34S-C129S, , (iii) D74E, D74Q or D74N, iv) Q11E, Y12F, M17L, Q22E and/or K81R; or any combination thereof, the amino acid numbering being as shown in SEQ ID NO: 1, which i) reduces affinity of the IL-7 variant for IL-7 receptor (IL-7R) in comparison to the affinity of wth-IL-7 for IL-7R, and ii) improves pharmacokinetics of the bifunctional molecule comprising the IL-7 variant in comparison with a bifunctional molecule comprising wth-IL-7.

In one example, the IL-7 variant comprises a group of amino acid substitutions selected from the group consisting of C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, and C47S-C92S and C34S-C129S (i.e., the amino acid numbering being as shown in SEQ ID NO: 1).

In another example, the IL-7 variant comprises an amino acid substitution selected from the group consisting of W142H, W142F and W142Y (i.e., the amino acid numbering being as shown in SEQ ID NO: 1).

In another example, the IL-7 variant comprises in the amino acid substitution selected from the group consisting of D74E, D74Q and D74N (i.e., the amino acid numbering being as shown in SEQ ID NO: 1).

Particularly, the IL-7 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2-15.

In one example, the binding moiety comprises a heavy chain constant domain, preferably a Fc domain, of a human IgG1, optionally with a substitution or a combination of substitutions selected from the group consisting of T250Q/M428L; M252Y/S254T/T256E + H433K/N434F; E233P/L234V/L235A/G236A + A327G/A330S/P331S; E333A; S239D/A330L/I332E; P257I/Q311; K326W/E333S; S239D/I332E/G236A; N297A; L234A/L235A; N297A + M252Y/S254T/T256E; K322A and K444A, preferably selected from the group consisting of N297A optionally in combination with M252Y/S254T/T256E, and L234A/L235A.

Particularly, the binding moiety comprises a heavy chain constant domain, preferably a Fc domain, of a human IgG4, optionally with a substitution or a combination of substitutions selected from the group consisting of S228P; L234A/L235A, S228P + M252Y/S254T/T256E.17 and K444A.

Preferably, the immune cell is a T cell, preferably an exhausted T cell.

In one example, the target is expressed by T cells and the binding moiety binds to a target selected from the group consisting of PD-1, CD28, CD80, CTLA-4, BTLA, TIGIT, CD160, CD40L, ICOS, CD27, OX40, 4-1BB, GITR, HVEM, Tim-1, LFA-1, TIM3, CD39, CD30, NKG2D, LAG3, B7-1, 2B4, DR3, CD101, CD44, SIRPG, CD28H, CD38, CXCR5, CD3, PDL2, CD4 and CD8.

Preferably, the target is expressed by T exhausted cells and the binding moiety binds to a target preferably selected from the group consisting of PD-1, CTLA-4, BTLA, TIGIT, LAG3 and TIM3.

In one example, the binding moiety is an antibody or an antigen fragment thereof, and the N-terminal of the IL-7 variant is fused to the C-terminal of a heavy or light chain constant domain of the antibody or

antibody fragment thereof, preferably to the C-terminal of the heavy chain constant domain, optionally via a peptide linker.

In another example, the IL-7 variant is fused to the binding moiety by a peptide linker selected from the group consisting of GGGGS (SEQ ID NO: 68), GGGGSGGGGS (SEQ ID NO: 67), GGGGSGGGGS (SEQ ID NO: 69) and GGGGSGGGGS (SEQ ID NO: 70), preferably is (GGGGS)₃ (SEQ ID NO: 70).

In one example, the molecule comprises a first monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a first heterodimeric Fc chain optionally via a peptide linker, said first heterodimeric Fc chain being covalently linked by the C-terminal end to the N-terminal end of the IL-7 variant, optionally via a peptide linker, and a second monomer comprising a complementary second heterodimeric Fc chain devoid of antigen-binding domain. Preferably, in the second monomer, the complementary second heterodimeric Fc chain covalently linked to the IL-7 variant, optionally via a peptide linker, preferably covalently linked by C-terminal end to N-terminal of the IL-7 variant, optionally via a peptide linker.

In another example, the molecule comprises a first monomer comprising an antigen-binding domain covalently linked by C-terminal end to N-terminal end of a first heterodimeric Fc chain, optionally via a peptide linker, said first heterodimeric Fc chain being devoid of IL-7 variant, and a second monomer comprising a complementary second heterodimeric Fc chain devoid of antigen-binding domain, said second heterodimeric Fc chain being covalently to the IL-7 variant, optionally via a peptide linker, preferably linked by C-terminal end to N-terminal of the IL-7 variant, optionally via a peptide linker.

In another example, the molecule comprises a first monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a first heterodimeric Fc chain optionally via a peptide linker, and a second monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a complementary second heterodimeric Fc chain optionally via a peptide linker, wherein only one of heterodimeric Fc chains, preferably the first one, is covalently linked by the C-terminal end to the N-terminal end of the IL-7 variant.

Particularly, the antigen-binding domain of the bifunctional molecule is a Fab domain, a Fab', a single-chain variable fragment (scFV) or a single domain antibody (sdAb).

Preferably, the antigen-binding domain comprises or consists essentially of: (i) a heavy chain comprising a CDR1 of SEQ ID NO: 51, a CDR2 of SEQ ID NO: 53 and a CDR3 of SEQ ID NO: 55, 56, 57, 58, 59, 60, 61 or 62; and (ii) a light chain comprising a CDR1 of SEQ ID NO: 64 or SEQ ID NO: 65, a CDR2 of SEQ ID NO: 66 and a CDR3 of SEQ ID NO: 16.

Particularly, the antigen-binding domain comprises or consists essentially of:

(a) a heavy chain variable region (VH) comprising or consisting of an amino acid sequence of SEQ ID NO: 18, 19, 20, 21, 22, 23, 24 or 25;

(b) a light chain variable region (VL) comprising or consisting of an amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 28.

Preferably, the antigen-binding domain comprises or consists essentially of a heavy chain variable region (VH) of SEQ ID NO: 24 and a light chain variable region (VL) of SEQ ID NO: 28.

In another aspect, the disclosure also relates to an isolated nucleic acid sequence or a group of isolated nucleic acid molecules encoding the bifunctional molecule according to the disclosure.

- 5 In yet another aspect, the disclosure also concerns a host cell comprising the isolated nucleic acid according to the disclosure.

In yet another aspect, the disclosure also concerns a pharmaceutical composition comprising the bifunctional molecule, the nucleic acid or the host cell according to the disclosure, optionally with a pharmaceutically acceptable carrier.

- 10 In a further aspect, the disclosure concerns the molecule as disclosed herein, the nucleic acid as disclosed herein or the host cell as disclosed herein, or the pharmaceutical composition as disclosed herein, for use in therapy.

- In a further aspect, the disclosure concerns a use of the molecule as disclosed herein, the nucleic acid as disclosed herein or the host cell as disclosed herein, or the pharmaceutical composition as disclosed
15 herein in the manufacture of a medicament for treating cancer or an infectious disease.

The disclosure finally concerns the bifunctional molecule, the nucleic acid, the host cell or the pharmaceutical composition according to the disclosure, for use as a medicament, especially for use in the treatment of a cancer or an infectious disease.

BRIEF DESCRIPTION OF THE DRAWINGS

- 20 **Figure 1: PD-1 binding ELISA assay.** Human recombinant PD-1 (rPD1) protein was immobilized and antibodies were added at different concentrations. Revelation was performed with an anti-human Fc antibody coupled to peroxidase. Colorimetry was determined at 450 nm using TMB substrate. **A.** PD-1 binding of the bifunctional molecule comprising an anti-PD1 antibody and an IL-7 mutated on the amino acid D74, Q22, M17, Q11, K81. **B.** PD-1 binding of the bifunctional molecule comprising an IL-7 mutated
25 on the amino acid W142 **C.** PD-1 binding of the bifunctional molecule mutated in the disulfide bonds of IL-7 (SS1, SS2 and SS3 mutant). All molecules tested in this figure were constructed with an IgG4m isotype and a GGGGSGGGSGGGGS linker between the Fc and IL-7 domain.

- Figure 2: CD127 binding ELISA assay of IgG fused mutated IL-7.** PD-1 recombinant protein was immobilized on the plate, then bifunctional anti-PD-1 IL-7 molecules were preincubated with CD127
30 recombinant protein (Histidine tagged, Sino ref 10975-H08H) and added to the well. Revelation was performed with a mixture of an anti-histidine antibody coupled to biotin + streptavidin coupled to Peroxidase. Colorimetry was determined at 450 nm using TMB substrate. **A.** CD127 binding of the bifunctional molecule comprising IL-7 mutated on the amino acid D74, Q22, M17, Q11, K81. **B.** CD127 binding of the bifunctional molecule comprising IL-7 mutated on the amino acid W142.

Figure 3: IL-7R signaling pathway of the different bifunctional molecules as measured by STAT5 phosphorylation. Human PBMCs isolated from peripheral blood of healthy volunteers were incubated 15 minutes with bifunctional anti-PD-1 IL-7 molecules. Cells were then fixed, permeabilized and stained with an AF647 labeled anti-pSTAT5 (clone 47/Stat5(pY694)). Data were obtained by calculating MFI pSTAT5 in CD3 T cells. **A.** pSTAT5 activation of the anti-PD-1 IL-7 bifunctional molecule comprising an IL-7 mutated on the amino acid D74, Q22, M17, Q11, K81. **B.** pSTAT activation of the anti PD-1 IL-7 bifunctional molecule comprising an IL-7 mutated on the amino acid W142 **C.** pSTAT5 activation of the anti PD-1 IL-7 bifunctional molecule comprising an IL-7 mutated in the disulfide bonds of IL-7 , SS2 (● black) and SS3 (▲) in

CLAIMS

1. A bifunctional molecule comprising an interleukin 7 (IL-7) variant conjugated to a binding moiety, wherein:

- the binding moiety binds to a target specifically expressed on immune cells surface,

5 - the IL-7 variant presents at least 75% identity with a wild type human IL-7 (wth-IL-7) comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 1, wherein the variant comprises at least one amino acid mutation selected from the group consisting of (i) W142H, W142F or W142Y, (ii) C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, or C47S-C92S and C34S-C129S, , (iii) D74E, D74Q or D74N, iv) Q11E, Y12F, M17L, Q22E and/or K81R; or any combination thereof, the amino acid numbering
10 being as shown in SEQ ID NO: 1, which i) reduces affinity of the IL-7 variant for IL-7 receptor (IL-7R) in comparison to the affinity of wth-IL-7 for IL-7R, and ii) improves pharmacokinetics of the bifunctional molecule comprising the IL-7 variant in comparison with a bifunctional molecule comprising wth-IL-7.

2. The molecule according to claim 1, wherein the IL-7 variant comprises an amino acid substitution selected from the group consisting of W142H, W142F and W142Y, the amino acid numbering being as
15 shown in SEQ ID NO: 1.

3. The molecule according to claim 1 or 2, wherein the IL-7 variant comprises a group of amino acid substitutions selected from the group consisting of C2S-C141S and C47S-C92S, C2S-C141S and C34S-C129S, and C47S-C92S and C34S-C129S, the amino acid numbering being as shown in SEQ ID NO: 1.

4. The molecule according to any one of claims 1-3, wherein the IL-7 variant comprises in the amino acid
20 substitution selected from the group consisting of D74E, D74Q and D74N, the amino acid numbering being as shown in SEQ ID NO: 1.

5. The molecule according to any one of claims 1-4, wherein the IL-7 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2-15.

6. The molecule according to any one of claims 1-5, wherein the binding moiety comprises a heavy chain
25 constant domain, preferably a Fc domain, of a human IgG1, optionally with a substitution or a combination of substitutions selected from the group consisting of T250Q/M428L; M252Y/S254T/T256E + H433K/N434F; E233P/L234V/L235A/G236A + A327G/A330S/P331S; E333A; S239D/A330L/I332E; P257I/Q311; K326W/E333S; S239D/I332E/G236A; N297A; L234A/L235A; N297A + M252Y/S254T/T256E; K322A and K444A, preferably selected from the group consisting of N297A optionally in combination with
30 M252Y/S254T/T256E, and L234A/L235A.

7. The molecule according to any one of claims 1-5, wherein the binding moiety comprises a heavy chain constant domain, preferably a Fc domain, of a human IgG4, optionally with a substitution or a combination

of substitutions selected from the group consisting of S228P; L234A/L235A, S228P + M252Y/S254T/T256E.17 and K444A.

8. The molecule according to any one of claims 1-7, wherein the immune cell is a T cell, preferably an exhausted T cell.

5 9. The molecule according to claim 8, wherein the target is expressed by T cells and the binding moiety binds to a target selected from the group consisting of PD-1, CD28, CD80, CTLA-4, BTLA, TIGIT, CD160, CD40L, ICOS, CD27, OX40, 4-1BB, GITR, HVEM, Tim-1, LFA-1, TIM3, CD39, CD30, NKG2D, LAG3, B7-1, 2B4, DR3, CD101, CD44, SIRPG, CD28H, CD38, CXCR5, CD3, PDL2, CD4 and CD8.

10 10. The molecule according to claim 8, wherein the target is expressed by T exhausted cells and the binding moiety binds to a target preferably selected from the group consisting of PD-1, CTLA-4, BTLA, TIGIT, LAG3 and TIM3.

11. The molecule according to any one of claims 1-10, wherein the binding moiety is an antibody or an antigen fragment thereof, and the N-terminal of the IL-7 variant is fused to the C-terminal of a heavy or light chain constant domain of the antibody or antibody fragment thereof, preferably to the C-terminal of the heavy chain constant domain, optionally via a peptide linker.

12. The molecule according to claim 11, wherein the IL-7 variant is fused to the binding moiety by a peptide linker selected from the group consisting of GGGGS (SEQ ID NO: 68), GGGGSGGGGS (SEQ ID NO: 67), GGGGSGGGGS (SEQ ID NO: 69) and GGGGSGGGGSGGGGS (SEQ ID NO: 70), preferably is (GGGGS)₃.

13. The molecule according to any one of claims 1-12, wherein the molecule comprises a first monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a first heterodimeric Fc chain optionally via a peptide linker, said first heterodimeric Fc chain being covalently linked by the C-terminal end to the N-terminal end of the IL-7 variant, optionally via a peptide linker, and a second monomer comprising a complementary second heterodimeric Fc chain devoid of antigen-binding domain.

14. The molecule according to claim 13, wherein, in the second monomer, the complementary second heterodimeric Fc chain covalently linked to the IL-7 variant, optionally via a peptide linker, preferably covalently linked by C-terminal end to N-terminal of the IL-7 variant, optionally via a peptide linker.

15. The molecule according to any one of claims 1-12, wherein the molecule comprises a first monomer comprising an antigen-binding domain covalently linked by C-terminal end to N-terminal end of a first heterodimeric Fc chain, optionally via a peptide linker, said first heterodimeric Fc chain being devoid of IL-7 variant, and a second monomer comprising a complementary second heterodimeric Fc chain devoid of antigen-binding domain, said second heterodimeric Fc chain being covalently to the IL-7 variant,

optionally via a peptide linker, preferably linked by C-terminal end to N-terminal of the IL-7 variant, optionally via a peptide linker.

16. The molecule according to any one of claims 1-12, wherein the molecule comprises a first monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a first heterodimeric Fc chain optionally via a peptide linker, and a second monomer comprising an antigen-binding domain covalently linked via C-terminal end to N-terminal end of a complementary second heterodimeric Fc chain optionally via a peptide linker, wherein only one of heterodimeric Fc chains, preferably the first one, is covalently linked by the C-terminal end to the N-terminal end of the IL-7 variant.

17. The molecule according to any one of claims 13-16, wherein the antigen-binding domain is a Fab domain, a Fab', a single-chain variable fragment (scFV) or a single domain antibody (sdAb).

18. The molecule according to any one of claims 13-17, wherein the antigen-binding domain comprises or consists essentially of: (i) a heavy chain comprising a CDR1 of SEQ ID NO: 51, a CDR2 of SEQ ID NO: 53 and a CDR3 of SEQ ID NO: 55, 56, 57, 58, 59, 60, 61 or 62; and (ii) a light chain comprising a CDR1 of SEQ ID NO: 64 or SEQ ID NO: 65, a CDR2 of SEQ ID NO: 66 and a CDR3 of SEQ ID NO: 16.

19. The molecule according to any one of claims 13-17, wherein the antigen-binding domain comprises or consists essentially of:

(a) a heavy chain variable region (VH) comprising or consisting of an amino acid sequence of SEQ ID NO: 18, 19, 20, 21, 22, 23, 24 or 25;

(b) a light chain variable region (VL) comprising or consisting of an amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 28.

20. The molecule according to any one of claims 13-19, wherein the antigen-binding domain comprises or consists essentially of a heavy chain variable region (VH) of SEQ ID NO: 24 and a light chain variable region (VL) of SEQ ID NO: 28.

21. An isolated nucleic acid sequence or a group of isolated nucleic acid molecules encoding the bifunctional molecule according to any one of claims 1-20.

22. A host cell comprising the isolated nucleic acid according to claim 21.

23. A pharmaceutical composition comprising the bifunctional molecule according to any one of claims 1-20, the nucleic acid according to claim 21 or the host cell according to claim 22, optionally with a pharmaceutically acceptable carrier.

24. The molecule according to any one of claims 1-20, the nucleic acid according to claim 21 or the host cell according to claim 22, or the pharmaceutical composition according to claim 23, for use in therapy.

25. Use of the molecule according to any one of claims 1-20, the nucleic acid according to claim 21 or the host cell according to claim 22, or the pharmaceutical composition according to claim 23 in the manufacture of a medicament for treating cancer or an infectious disease.